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MEDICAL RESEARCH FUND

No. 10: A SUGGESTION AS TO THE CAUSE OF THE LESSENED PRODUCTION OF INDOL IN MEDIA CONTAINING GLUCOSE, by Annie Homer

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A SUGGESTION AS TO THE CAUSE OF THE LESSENED PRODUCTION OF INDOL IN MEDIA CONTAINING GLUCOSE.

By ANNIE HOMER,

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(*From the Laboratories of the University of Toronto.*)

In the *British Medical Journal*, Dec. 18, 1915, there is a letter by Mr Benians, in which he refers to the Toxic Bodies of the Bacillus of Malignant Oedema, and discusses the production of aromatic bodies by the action of bacteria on proteins. He also speculates as to the cause of the lessened production of indol in peptone culture media containing glucose, glycerine and other similar bodies. As the metabolic activities of some bacteria decompose these substances with the production of acid, Mr Benians wonders whether it is this acidity which so modifies the action of the proteolytic ferment that cultures, which, in the ordinary course of events are very foul remain sweet when glucose is present.

In this connection the following observations may be of interest even though, owing to unforeseen difficulties, the work as originally planned is not yet completed.

The experimental work detailed in this communication was undertaken with the object of ascertaining to what extent tryptophane is decomposed by various organisms into indolpropionic and indolacetic acids and into indol and skatol. The organisms were grown on a synthetic medium containing tryptophane and the production of the decomposition products of tryptophane was detected colorimetrically by means of the nitrite and the p. dimethyl amidobenzaldehyde tests.

In a previous communication I have shown that, in order to apply these colorimetric tests for the presence of indol, skatol, indolacetic and indolpropionic acids, it is necessary to subject the liquids containing them to a preliminary process of separation. Extraction of the solutions with ether will remove the soluble indol, skatol, indolacetic and indolpropionic acids from the ether insoluble tryptophane. A further separation may be effected by steam distillation of the ether extract: indol and skatol being carried over in the steam whereas indolacetic and indolpropionic acids remain behind. The individuals of these pairs can be distinguished from each other as, fortunately, they give different reactions with the colorimetric reagents employed.

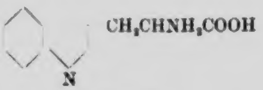
Two synthetic media were employed, the one A containing the necessary nutrient salts, 1 % of gelatine and 0·15 % of tryptophane, the other B containing in addition 1 % of glucose.

After sterilisation the tubes containing the media were inoculated with as many strains of aerobic organisms as were available and the various indol products were tested for colorimetrically.

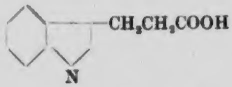
It was noticed that where growth took place it was more luxuriant in the medium A than in the medium B: in fact in several cases while there was growth in A there was none in B.

It was also observed that the indol or indolacetic acid production by the various organisms was greater in A than in B. This phenomenon may be due to one of two causes: either that the organisms find it easier to derive their energy from the decomposition of glucose than of tryptophane, or else that the glucose by chemically reacting with tryptophane produces a substance or substances not so readily useful as is tryptophane for bacterial metabolism.

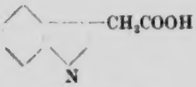
The chemical reactions involved in the formation of indol, skatol, indolpropionic and indolacetic acids from tryptophane necessitate the preliminary removal of the *amino* (NH_2-) group from the side chain of the molecule thus:



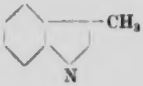
Tryptophane



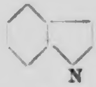
Indolpropionic acid



Indolacetic acid



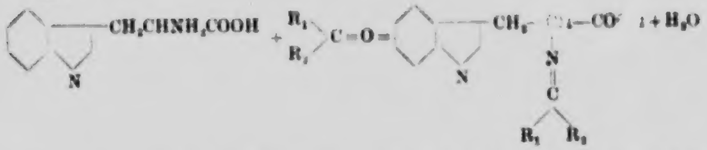
Skatol



Indol

Now, it has been shown that the *amino* group of tryptophane will readily react at body temperature with the *carbonyl* group of an aldehyde or of a ketone to form colourless compounds of the type:

Type I.



These compounds, of which several have been isolated and of which a few have been analysed (Homer, *Biochem. Journ.* 1913, VII, 101), are less readily attacked by various chemical reagents than tryptophane itself.

I therefore venture to suggest that in the medium containing glucose there is lessened indol production because of the formation of a glucose-tryptophane complex which is not so readily attacked as tryptophane alone. Moreover, in a medium in which the tryptophane is rendered chemically unsuitable for bacterial decomposition there is impaired growth of the organisms because, once the tryptophane is inactivated, gelatine alone cannot be regarded as a satisfactory substitute for the protein constituent of a culture medium. Gelatine lacks tryptophane, a substance which Hopkins and Willcock (*Journ. Physiol.* XXXV 88) and Osborne and Mendel (*Journ. Biol. Chem.* 1914, XVII, 325) have shown to be necessary for the maintenance of the life of the individual.

It was further noticed that in the course of the sterilisation the colour of B became more intense than that of A, and further, during the incubation period the intensity of colour of both media became more marked: A becoming yellow while B became dark brown, almost black, owing to the formation of the so-called "humin-like" substances. This intense browning of the colour was a special feature of the tryptophane-glucose combination as it did not take place in the media containing tryptophane and gelatine but no glucose, nor did it occur in media containing glucose and gelatine but no tryptophane.

Now, tryptophane and other indol compounds and pyrrol derivatives by virtue of their *imino* (-NH-) group will readily react with the *carbonyl* group of aldehydo- or keto-compounds to form condensation products of intense colour. The tryptophane and other indol complexes of this type vary in colour from yellow or red to brown or black (Homer, *loc. cit.*). Their constitution has not yet been determined but the available data seem to indicate that at least two indol nuclei are involved in the condensation. Thus in the case of indol:

Type II. $2C_8H_7N + 5HCHO = C_{21}H_{20}O_3N_2 + 2H_2O$.

The compounds of this type are markedly stable.

From these considerations it is probable that the production of the "humin-like" substance characteristic of the glucose-tryptophane medium B is due to the formation of an intensely coloured compound of Type II by the chemical interaction of glucose and tryptophane or of glucose and the previously mentioned glucose-tryptophane complex of Type I.

We thus see that in a liquid culture medium containing glucose and substances such as peptone, which on hydrolysis give rise to tryptophane, there is the possibility of the formation of two types of condensation products between glucose and tryptophane. The formation of a compound of what I have designated Type I is probably responsible for the lessened production of indol and other foul smelling indol substances and the formation of substances of Type II is probably the cause of the darkening during sterilisation and incubation.

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